Food Control 50 (2015) 173–183

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Wash water disinfection of a full-scale leafy vegetables washing process with hydrogen peroxide and the use of a commercial metal ion mixture to improve disinfection efficiency

S. Van Haute [a, b], I. Tryland [c], A. Veys [b], I. Sampers [b, *]

[a] Laboratory of Food Microbiology and Food Preservation, Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
[b] Laboratory of Food Microbiology and Biotechnology, Department of Industrial Biological Sciences, Faculty of Bioscience Engineering, Ghent University Campus Kortrijk, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium
[c] Norwegian Institute for Water Research, Gaustadalléen 21, No-0349 Oslo, Norway

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form
13 June 2014
Accepted 26 August 2014
Available online 3 September 2014

Keywords:
Leafy vegetables
Hydrogen peroxide
Metal ions
Water disinfection
Decontamination

ABSTRACT

Hydrogen peroxide (H_2O_2) was used to maintain the microbial wash water quality of a full-scale leafy vegetables (radicchio, sugar loaf, curled endive, lollo, lollo rosso) wash water process. Despite addition of 300 L/h of 1.8% H_2O_2 to a 450 L washing bath (333 ± 50 kg/h fresh-cut produce introduction speed), the H_2O_2 quickly decreased and a lower wash water contamination of aerobic psychrotrophic plate count (APC) and enterococci than without addition of H_2O_2 could not be maintained. There was no significant difference between the APC on fresh-cut leafy vegetables washed with H_2O_2 and those washed with water.

In a second part, lab-scale experiments were performed to assess the impact of a commercial metal ion formulation (Bacsan®, containing a. o. Cu^{2+}, Zn^{2+}, Ag^{+}) on the stability of H_2O_2 in artificial wash water, made from iceberg lettuce and tap water. Bacsan improved the stability of H_2O_2 in artificial lettuce wash water and fresh-cut leafy vegetables wash water from a processing company and synergistically increased the disinfection efficiency of APC and *Escherichia coli* (*E. coli*) compared to H_2O_2 or Bacsan. Increasing chemical oxygen demand (COD) had detrimental effect on the H_2O_2 stability and disinfection efficiency. Addition of Ag^{+} to Bacsan further synergistically enhanced the H_2O_2 stability.

H_2O_2 is not suited as an *in situ* wash water disinfectant to avoid cross-contamination in fresh-cut leafy vegetables washing processes due to the slow water disinfection kinetics and the rapid H_2O_2 consumption. However, H_2O_2/Bacsan shows potential for use in off-line processes.

1. Introduction

Among fresh produce, leafy vegetables are one of the commodities most frequently implicated with food disease outbreaks, the culprit most often being *Escherichia coli* O157: H7 or *Salmonella* spp. (Olaimat & Holley, 2012; Tomas-Callejas et al., 2012). Washing of fresh-cut lettuce is often the only processing step able to reduce the microbial load (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009). Current washing treatments with the purpose of decontaminating fresh-cut produce for microbial safety or quality reasons, have evolved from processes that were originally developed to remove soil from whole produce, to a water disinfection process for removal of microbial targets from fresh-cut produce (Sapers, 2001). The success of these washing processes to remove naturally present microorganisms from fresh-cut produce is limited (1–3 log reduction), i.e. microbial reductions occur but total removal cannot be achieved. The access of sanitizers to the target microorganisms is hindered by the presence of microorganisms in biofilms, attachment near and within stomata, and internalization through cut surfaces and other tissue wounds. Therefore it is preferable to avoid contamination wherever possible by implementing good agricultural and manufacturing practices during the production and processing of fresh produce (Holvoet, Jacxsens, Sampers, & Uyttendaele, 2012; Holvoet, Sampers, Callens, Dewulf, & Uyttendaele, 2013; Keskinen, Burke, & Annous, 2009; López-Gálvez, Gil, Truchado, Selma, & Allende, 2010; Sapers, 2001). The post-harvest washing water is a vehicle for microbial cross-contamination and to counter this an *in situ* wash

* Corresponding author. Tel.: +32 56 24 12 11; fax: +32 56 24 12 24.
E-mail address: imca.sampers@ugent.be (I. Sampers).

http://dx.doi.org/10.1016/j.foodcont.2014.08.028
0956-7135/

water disinfection can be performed. Water disinfection can also be used to treat the wash water before reusing it (i.e. reconditioning) for a similar or different purpose. The efficiency of wash water disinfection is not limited by the issues that plague decontamination, but the effectiveness of chemical oxidants (a. o. chlorine, chlorine dioxide, ozone, H_2O_2, peracetic acid) is hindered by the presence of organic matter in the wash water, the degree depending on the properties of the chemical oxidant (Van Haute, Sampers, Jacxsens, & Uyttendaele, 2013).

H_2O_2 does not produce toxic fumes in the worker space and is an environmentally friendly alternative to chlorine for decontamination of fresh produce, as it breaks down in water and oxygen (Tofant, Vucemilo, Pavicic, & Milic, 2006), and does not form carcinogenic disinfection byproducts (USEPA, 1997; Van Haute, Sampers, Jacxsens, et al., 2013). Considerable research has been conducted on the use of H_2O_2 as produce decontamination agent against bacterial and viral indicator organisms, pathogenic bacteria, or spoilage microflora on fresh (-cut) fruit and vegetables (Parish et al., 2003; Ukuku, Bari, & Kawamoto, 2012), among which some experiments have been performed on leafy vegetables (Allwood, Malik, Hedberg, & Goyal, 2004; Hadjok, Mittal, & Warriner, 2008; Huang & Chen, 2011; Li et al., 2011; Lin, Moon, Doyle, & McWatters, 2002). On the contrary, its use as a water disinfectant to control the wash water quality of fresh produce washing processes is virtually unexplored. Earlier water disinfection studies that focused on inactivating vegetative bacteria, bacterial spores, viruses, or protozoa have shown that H_2O_2 by itself is a slow acting water disinfectant, requiring high dosages and contact times for microbial inactivation (Barbee, Weber, Sobsey, & Rutala, 1999; Raffellini, Guerrero, & Alzamora, 2008; Raffellini, Schenk, Guerrero, & Alzamora, 2011; Toledo, Escher, & Ayres, 1973; Weir, Pokorny, Carreno, Trevors, & Lee, 2002). Combined with Ag^{+} and $Cu^{1\text{ or }2+}$, performance of H_2O_2 can be enhanced (Batterman, Zhang, & Wang, 2000; Orta De Velasquez, Yanez-Noguez, Jimenez-Cisneros, & Luna Pabello, 2008; Pedahzur et al., 2000; Pedahzur, Shuval, & Ulitzur, 1997).

In this study, the use of H_2O_2 to maintain the microbial wash water quality in a full-scale industrial fresh-cut leafy-vegetables washing process was assessed. To the knowledge of the authors, this is the first published study that utilizes H_2O_2 as wash water sanitizer in a full-scale washing process of fresh-cut leafy vegetables. Also, lab-scale experiments were performed to assess the use of Bacsan (containing a. o. Cu^{2+}, Ag^{+}, and Zn^{2+}) to improve the H_2O_2 disinfection efficiency in post-harvest water disinfection processes.

2. Materials and methods

2.1. Water disinfection in a fresh-cut leafy vegetables processing company

2.1.1. Experimental setup

Experiments were executed in a Belgian fresh-cut leafy vegetables processing company. First, a run was executed without addition of water disinfectant, i.e. the 'blank' run. A batch of 400 kg mixed salad was processed, containing radicchio (33%), sugar loaf (*Chicorium intybus*) (33%) and curled endive (33%). The leafy vegetables were cut (in pieces of 1 by 5 cm), and transported through two subsequent immersion washing baths (washing bath 1: WB1 and washing bath 2: WB2) with a volume of 450 L each, and a leafy vegetable residence time of 1 min in each washing bath. The washing system consisted of bubble washers, i.e. production of agitation in the washing baths by air bubble injection through underwater air nozzles. Subsequently they were transported by a conveyer belt to a centrifuge for dewatering, followed by a weighing unit (computer controlled weight proportioning scales). Both washing baths were filled with bore hole water, cooled on beforehand to 2 °C. During the washing process, 300 L/h of bore hole water was added to each of the washing baths. Wash water was recirculated within washing baths but not between washing baths. The only water that was transferred from WB1 to WB2 was the water that was attached to the transferred lettuce. Two wash water disinfection experiments were performed. In both experiments, the same types of leafy vegetables were processed during the wash water disinfection experiments of which the first batch (467 ± 55 kg) was the same leafy vegetables mix as in the blank runs. In addition, a second batch (258 ± 31 kg) was processed, consisting of white lollo (*Lactuca sativa* cv. Lollo Bianco) (50%) and lollo rosso (*L. sativa* cv. Lollo Rosso) (50%). For each type of leafy vegetable and experiment, the crops originated from the same farm, and the crops were processed at the day of harvest. On average leafy vegetables were washed at 333 ± 50 kg/h. In the disinfection experiments, WB1 was operated identically to the blank runs. In the first disinfection experiment, WB2 was filled with 1.8% H_2O_2 (i.e. 4% EcoClearProx, ABT Belgium, Belgium) and 300 L/h 1.8% H_2O_2 of bore hole water was added. In the second disinfection experiment, WB2 was filled with 1.8% H_2O_2 and 300 L/h of bore hole water was added. During processing, 300 L/h of wash water was tapped from the washing bath and 5.4 L/h H_2O_2 was dosed (again to obtain addition of 1.8% H_2O_2/L) and sent through a low pressure UV-C system (Aquada 2, Wedeco, Belgium; 55 W) with fluence of 240 mJ/cm^2 at a flow of 300 L/h and 98% UV 254 nm transmittance/cm, before recirculation to WB2.

2.1.2. Sampling in the fresh-cut leafy vegetable processing company

Samples of the fresh-cut leafy vegetables, water samples from WB1 and WB2, and samples from the food contact surfaces of the conveyer belt and the weighing unit were taken five times throughout the processing: at the start of batch 1, at the middle of batch 1, at the end of batch 1 = start of batch 2, in the middle of batch 2, at the end of batch 2. About 250 g of fresh-cut leafy vegetables was sampled and put directly into a sterile stomacher bag. For sampling the raw material, each lettuce type was sampled separately per batch, and averaged as the microbial count of the raw material. The water samples were collected into a sterile 1 L bottle according to ISO 19458:2006 (ISO, 2006). Excess H_2O_2 was quenched with sterile $Na_2S_2O_3$. The food contact surfaces were sampled with sterile swabs. Aseptic templates covering 50 cm^2 were used and a sterile swab moistened in 5 mL of buffered peptone water was used to swab a delimited area vertically, horizontally, and diagonally. All the samples were stored and transported in the dark at <4 °C to the lab for further handling and subsequent microbial analysis within 12 h. For each measuring point two independent samples were taken. At each time point and operation unit, water and food contact surfaces were sampled at two consistent points, and each of the two samples for raw materials screening originated from two crops.

2.1.3. Microbial analyses

For the fresh-cut leafy vegetables samples and food contact surfaces, APC and *E. coli* were enumerated, whereas in the water also enterococci were enumerated. For the fresh-cut leafy vegetables samples, 10 g of fresh-cut leafy vegetables was weighed in a stomacher bag and homogenized for 1 min in 90 mL buffered peptone water. The enumeration of APC was done with the reference method ISO 4833:2003 (ISO, 2003), with the exception that the plates were incubated at 22 °C for five days instead of at 30 °C for 3 days. *E coli* was enumerated with the pour plate method on RAPID'*E.coli 2* agar (BioRad, France), a selective chromogenic medium, incubated for 24 h at 37 °C. For the water and food contact